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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/009,782	03/25/2002	Ken-Ichi Takeuchi	217301US0PCT	1319

22850 7590 08/26/2003

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EXAMINER
WALICKA, MALGORZATA A

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 08/26/2003

12

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application N .	Applicant(s)	
	10/009,782	TAKEUCHI ET AL.	
	Examiner	Art Unit	
	Malgorzata A. Walicka	1652	

-- The MAILING DATE of this communication appears on the c ver sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 May 2003 and 04 June 2003* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *14-34* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *14-34* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *sequence alignment* .

The Amendment, Request for Reconsideration and Statement filed on may 21, 2003 as paper No. 9, and Supplemental Amendment comprising the substitute specification, filed as paper No. 10 on June 4, 2003 are acknowledged.

The amendment to the claims and the substitute specification have been entered as requested. Claims 1-13 are canceled. New claims 14 - 34 are entered. Claims 14-34 are pending and are the subject of this Office Action.

DETAILED ACTION

1. Objections

The objection to the specification is withdrawn, because the substitute specification has been filed.

The amendment filed on June 4, 2003 is objected to under 35 U.S.C. 132 because it introduces new matter into the disclosure. 35 U.S.C. 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is at page 11, line 11 the name of the restriction endonuclease Sau3A1.

Applicant is required to cancel the new matter in the reply to this Office Action.

Objections to claims 4 and 11 are moot because the claims have been cancelled.

In the newly add claim 19 the word "by" should be added after the word modified.

Formal drawings are acknowledged.

2. Rejections

3.1. 35 USC, section 112, second paragraph

Rejection of claims claim 1-13 under 35 U.S.C. 112, second paragraph made in the previous Office Action is moot, because the calms have been cancelled.

Claim 21 and 22 recite the limitation "the zinc tolerance " in the fist line. There is insufficient antecedent basis for this limitation in the claim, because the base claim 14 is reciting the phrase "wherein said microorganism is zinc resistant."

Claim 19 and 20 are indefinite, because they do not identify the D-aminoacylase producing gene that was modified. In addition, claim 20 is not clear as to when the D-aminoacyklase gene was modified. Was the gene modified before transformation or thereafter?

Claims 14, 17-27 and 28-34 are rejected because they are confusing. The claims recite or are directed to a nucleic acid sequence encoding the amino acid SEQ ID NO: 2, wherein said DNA molecule comprises the following sequence of restriction sites: EcoRI-BglII-PvuII-HindIII or SalI-BglII-PvuII. Plasmid pKNSD2 presented in Fig. 2, comprises the restriction site EcoRI before the start codon of the gene for D-aminoacylase, as well as the restriction sites EcoRV and Hind III in 3' noncoding portion the gene inserted in the plasmid (residues 1605 and 1753), and in addition the open reading frame itself comprises SalI, BglII and

PvuII (residues 190, 284, and 1296, respectively) recognition sites. However, those recognition sites do not compose the sequence of nucleotides described as EcoRI-BglII-PvuII-HindIII, i.e., GAATTCAGATCTCAGCTGAAGCTT, because they occur in the different parts of the DNA constructs and ORF.

2.2. 35 USC, section 112, first paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2.1.1. Lack of written description

Claims 14-27 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are directed to a zinc-tolerant microorganism transformed with a gene encoding D-aminoacylase from *Alcaligenes*, and the use of such organism for production of D-aminoacylase, wherein **the expression of said gene is enhanced in the presenc of zinc ions.** The claims are directed to a

large genus of microorganisms and a large genus of methods of producing the enzyme. Applicants, however, failed to describe any representative species of such genera of microorganism and methods, because Applicants did not disclose a zinc resistant microorganisms containing a D-aminoacylase gene from *Alcaligenes* wherein **the expression of D-aminoacylase gene is increased** by the presence of zinc ions. Applicants disclosed a zinc resistant transformant *E. coli* that overexpresses D-aminoacylase of *Alcaligenes xylosoxydans* because the DNA construct used for transformation was designed to cause such overexpression, i.e. the ribosome binding site was modified, the *tac* inducible promoter was used, and the Hind III recognition site was introduced.

Applicant measured the activity of the enzyme directly in the *E. coli* culture medium supplemented with zinc or zinc free, and found that the medium from the culture of *E.coli* exhibited higher enzyme activity when the zinc ions were present.

On page 14 Applicants write, "the enzyme activity in the 0.2 mM zinc-added culture medium was 58.86 U/mL (broth –out pH of 5.3) and the enzyme activity in the 2.0 mM zinc–added culture medium was 109.79 U/mL (broth-out pH of 5.11), compared with the enzyme activity of 21.78 U/mL in the zinc-free culture medium (broth-out pH of 5.05). Thus, it has been confirmed that the addition of zinc ion, at least within a predetermined concentration range, greatly improves the D-aminoacylase producing potency".

However, the phrase: "addition of zinc ion, at least within a predetermined concentration range, greatly improves the D-aminoacylase producing potency"

has no support in the Applicants disclosure. The reason is that the applicants did not provide any measurements of the number of D-aminoacylase molecules, or the D-aminoacylase mRNA content per cell of transformant, when it was cultivated in the presence or absence of zinc in the medium. Any of these measurements is necessary to conclude that the expression of the enzyme was increased.

D-aminoacylase is a zinc dependent metalloprotease whose activity is increased in the medium supplemented with zinc ions (see, for example, the paper by Wakayama et al. Role of conserved histidine residues in D-aminoacylase from *Alcaligenes xylosoxydans* subsp. *xylosoxydans* A-6", Bioscience, Biotechnology and Biochemistry, 2000, vol. 64, 1-8, on which the Applicants are co-authors; the article is included in the IDS). Thus, one skilled in the art would expect that the activity of the enzyme in the presence of 2 mM or 5 mM zinc ions containing medium would be higher than in the medium without zinc.

In conclusion, Applicants did not provide a sufficient description of the claimed invention so that one skilled in the art was convinced that at the time the application was filed applicants were in possession of the claimed invention.

Claims 14, 17-27 and 32-34 are rejected for lack of written description of the structure of the DNA molecule from *Alcaligens encoding* D-aminoacylase, said molecule comprising the following sequence of restriction sites: EcoRI-BglII-PvuII-HindIII or SalI-BglII-PvuII.

Applicants' invention is directed to a genus of transformed microorganisms or DNA molecules encoding enzymes originating from the genus *Alcaligens.* Said genus comprises many species. Applicants disclose only one species of said genus of enzymes, i.e. D-aminoacylase from *Alcaligenes xylosoxydans* subsp. *xylosoxydans* A-6 identified by SEQ ID NO: 2, however this is not sufficient to provide identifying structural characteristics of all the species of the genus, especially because the specification fails to teach any structure/function relationship for the disclosed species of the DNA molecule encoding D-aminoacylase from *Alcaligenes xylosoxydans* subsp. *xylosoxydans* A-6 identified by SEQ ID NO:2. Those skilled in the art realize that even a change of a single nucleotide in the encoding sequence can inactivate or change the kind of activity of the in the enzyme. Therefore, because the specification lacks description of the nucleotide changes which are neutral for the function of the encoded protein,

One skilled in the relevant art is not convinced that the inventor(s), at the time the application was filed, had possession of the claimed invention.

2.2.2. Scope of enablement

Rejection of claims 1-13 made in the previous Office Action for scope of enablement is moot because the claim have been cancelled.

However, new claims 14, 17-28 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a microorganism comprising DNA molecule encoding D-aminoacylase from *Alcaligenes xylosoxydans* subsp. *xylosoxydans* A-6 identified by SEQ ID NO: 2, does not

reasonably provide enablement for a transformed microorganism comprising DNA molecule encoding D-aminoacylase from the genus of *Alcaligenes,* said molecules comprising a sequence of the restriction sites EcoRI-BglII-PvuII-HindIII or SalI-BglII-PvuII, wherein expression of said DNA molecule increases in the presence of zinc ions.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. The claims are directed to a large genus of transformed microorganisms for which expression of DNA encoding D-aminoacylase form the genus *Alcaligens* is increased in the presence of zinc. Neither such molecules or transformants are disclosed by Applicants; see the above rejection for lack of written description.

The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Otherwise, undue experimentation is necessary to make the claimed invention.

Factors to be considered in determining whether undue experimentation is required are summarized *In re* Wands [858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)]. The Wands factors are: (a) the quantity of experimentation necessary, (b) the amount of direction or guidance presented, (c) the presence or absence of working example, (d) the nature of the invention, (e) the state of the prior art, (f) the relative skill of those in the art, (g) the predictability or unpredictability of the art, and (h) the breadth of the claim.

The nature and breadth of the claimed invention encompasses transformants comprising DNA molecules from the genus of *Alcaligenes*, wherein said molecules comprises a sequence of the restriction sites EcoRI-BglII-PvuII-HindIII or SalI-BglII-PvuII, or any microorganism resistant to zinc and expressing said DNA molecule, wherein expression of said molecule is increased in the presence of zinc ions.

Although the art of cloning, engineering, and expressing of genes and transforming microorganisms is well developed, and skills of those in the art high, the predictability of the results of transforming a zinc resistant microorganism with a DNA molecule encoding D-aminnoacylase comprising a sequence of the restriction sites EcoRI-BglII-PvuII-HindIII or SalI-BglII-PvuII, wherein said DNA molecule originates from any species of the *Alcaligens* genus, so that the expression of said gene was increased in the presence of zinc ions in the culture medium, is low. The specification does not teaches DNA molecules from the genus *Alcaligens* encoding D-aminoacylase whose expression may be increased by the presence of zinc in the medium. Thus, lack of this teaching forces one skilled in the art to do research outside the realm of routine experimentation. This experimentation has a low probability of success absent the guidance as to which particular structural feature of the genus of the DNA molecules is responsible for increase of expression of D-aminoacylase by zinc. Furthermore, the specification does not disclose any expression controlling element whose activity *in vitro* is increased by zinc, nor the specification teaches any expression vector containing a gene encoding D-aminoacylase, wherein said vector after

being transected to any zinc resistant microorganism exhibits higher expression when said transformant is cultivated in the presence of zinc. Thus, Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. Without further guidance on the part of Applicants how to select a DNA molecule from *Alcaligens* or to make an expression controlling element whose activity is increased in the presence of zinc, the experimentation left to those skilled in the art is unnecessary, improperly extensive and undue.

In addition, claims 28 - 34 are rejected because while the specification is enabling for the DNA molecule of SEQ ID NO: 1 encoding the amino acid sequence of SEQ ID NO: 2 plasmid pKNSD2 comprising SEQ ID NO: 1, does not reasonably provide enablement for a DNA molecules encoding D-aminoacylase from the genus *Alcaligens*, wherein said DNA molecule comprises a sequence of the restriction sites EcoRI-BglII-PvuII-HindIII or SalI-BglII-PvuII.

The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Otherwise, undue experimentation is necessary to make the claimed invention.

The nature and breadth of the claimed invention encompasses DNA molecules from the genus of *Alcaligenes*, wherein said molecules comprise the sequence of the restriction sites EcoRI-BglII-PvuII-HindIII or SalI-BglII-PvuII, and encode D-aminoacylase.

Although the art of cloning of genes, expressing them, and checking the enzymatic activity of the expressed proteins and making restriction maps and

sequencing the encoding DNA is well developed, and skills of those in the art high, selecting a DNA molecule from any species of the genus *Alcaligens, wherein said DNA molecule* encodes D-aminnoacylase and comprises the sequence of the restriction sites EcoRI-BglII-PvuII-HindIII or SalI-BglII-PvuII is out of routine experimentation. The specification does not teach other species of the genus *Alcaligens,* wherein said species would be the proper source of the claimed DNA molecules. Lack of this teaching forces one skilled in the art to do research outside the realm of routine experimentation. Thus, because Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims, the experimentation left to those skilled in the art is unnecessary, improperly extensive and undue.

2.3. 35 USC section 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 28, 29 and 32-34 are rejected under 35 U.S.C. 102(b) as being anticipated by Wakayama et al. (Cloning and Sequencing of a Gene Encoding D-Aminoacyase from *Alcaligens xylosoxydans* subsp. *xylosoxydans* A-6 and Expression of the Gene in *Escherichia coli*, Biosci. Biotech. Biochem, **1995**, 59, 2115-2119, included in IDS).

Te claims are directed to an isolated nucleic acid sequence which encodes the amino acid sequence of SEQ ID NO: 2.

Wakayama et al. disclose the DNA molecule that encodes the amino acid sequence of SEQ ID NO: 2; see Figure 3, page 2117 of the article, and the enclosed sequence alignment.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka, Ph.D., whose telephone number is (703) 305-7270. The examiner can normally be reached Monday-Friday from 10:00 a.m. to 4:30 p.m.

If attempts to reach examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, Ph.D. can be reached on (703) 308-3804. The fax number for this Group is (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionists whose telephone number is (703) 308-0196.

Malgorzata A. Walicka, Ph.D.

Art Unit 1652

Patent examiner

REBECCA E. PROUTY
PRIMARY EXAMINER
GROUP ~~1300~~ 1600

GenCore version 5.1.4_p5_4578

OM nucleic - protein search, using frame_plus_n2p model

Run on: May 11, 2003, 12:05:25 ; Search time 54.5 Seconds
(without alignments)
6201.993 Million cell updates/sec

Title: US-10-009-782-1
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Delop 6.0 , Delext 7.0

Searched: 283224 seqs, 96134422 residues

Total number of hits satisfying chosen parameters: 566448

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Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

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2: pir2:*
3: pir3:*
4: pir4:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	2511	76.1	484	2	JC4394	aminoacylase (EC 3
2	1000.5	30.3	488	2	JC4165	N-acyl-D-glutamate
3	942	28.6	526	2	B75202	d-aminoacylase (as
4	423.5	12.8	1106	2	JQ0405	hypothetical 119.5
5	407	12.3	581	2	B87678	hypothetical prote
6	338.5	10.3	529	2	T45134	hypothetical prote
7	337	10.2	924	2	S27923	gene LF3 protein -
8	321.5	9.7	660	1	QQBE3	BHLF1 protein - hu
c 9	312	9.6	660	1	QQBE3	BHLF1 protein - hu
10	307	9.3	680	2	S21323	probable endogluca
11	296.5	9.0	1367	1	S48478	glucan 1,4-alpha-g
c 12	296	9.1	1791	2	T02345	hypothetical prote
13	294	8.9	611	2	D70928	hypothetical prote
14	289.5	8.8	3020	2	A43932	mucin 2 precursor,
c 15	287.5	8.9	1106	2	JQ0405	hypothetical 119.5
16	284.5	8.6	1733	1	B45344	probable nuclear a
17	282.5	8.6	437	2	C39135	hypothetical prote
18	282.5	8.6	1460	1	EDBEIF	immediate-early pr
19	277.5	8.4	494	2	G84348	hypothetical prote
c 20	274	8.4	1733	1	B45344	probable nuclear a
21	273.5	8.3	1027	2	S28774	collagen alpha cha
22	272.5	8.3	1414	1	S23809	collagen alpha 2(I
23	270.5	8.2	507	2	T44768	antifreeze glycope
24	270	8.2	1464	1	CGHU1S	collagen alpha 1(I
c 25	267.5	8.2	1958	2	B40505	hypothetical prote
26	267	8.1	825	2	JC4163	DNA-binding protei
27	265.5	8.0	1791	2	T02345	hypothetical prote
28	265.5	8.0	3570	2	T45025	mucin MUC5B, trach
29	264.5	8.0	1466	1	CGHU7L	collagen alpha 1(I
30	264	8.0	1464	2	S59856	collagen alpha 1(I
31	263	8.0	1188	2	S49915	extensin-like prot
32	263	8.0	1453	2	S21626	collagen alpha 1(I
33	262	7.9	1344	1	A35175	mucin 1 precursor,
34	262	7.9	1958	2	B40505	hypothetical prote
c 35	261.5	8.1	640	2	T08179	LRG5 protein - Chl
36	261	7.9	779	1	CGBO1S	collagen alpha 1(I
37	261	7.9	839	2	F75518	hypothetical prote
38	260.5	7.9	580	2	T43481	probable mucin DKF
39	260.5	7.9	964	1	CGCH2S	collagen alpha 2(I
40	260.5	7.9	1042	1	CGCH1S	collagen alpha 1(I
41	258.5	7.8	2796	2	JC4743	fatty-acid synthas
42	255.5	7.7	1151	2	T18535	high molecular mas
c 43	253	7.8	738	2	E87627	hypothetical prote
44	251	7.6	1419	2	A41182	collagen alpha 1(I
45	251	7.6	1487	2	B41182	collagen alpha 1(I

ALIGNMENTS

RESULT 1
JC4394
aminoacylase (EC 3.5.1.14) - Alcaligenes xylosoxydans subsp. xylosoxydans A-6
N;Alternate names: N-acyl-D-amino acid amidohydrolase
C;Species: Alcaligenes xylosoxydans subsp. xylosoxydans A-6
C;Date: 20-Jan-1996 #sequence_revision 19-Apr-1996 #text_change 13-Sep-1998
C;Accession: JC4394
R;Wakayama, M.; Katsuno, Y.; Hayashi, S.; Miyamoto, Y.; Sakai, K.; Moriguchi, M.
Biosci. Biotechnol. Biochem. 59, 2115-2119, 1995
A;Title: Cloning and sequencing of a gene encoding D-aminoacylase from Alcaligenes
A;Reference number: JC4394; MUID:96100942; PMID:8541651
A;Accession: JC4394
A;Molecule type: DNA
A;Residues: 1-484 <WAK>
C;Comment: This enzyme, which catalyzes the hydrolysis of N-acyl derivatives ofneut
resence of zinc ion or EDTA.
C;Genetics:
A;Gene: dan
C;Superfamily: aminoacylase
C;Keywords: hydrolase
F;68-70/Region: zinc binding

Alignment Scores:

Pred. No.:	1.64e-128		
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Best Local Similarity:	100.00%	Conservative:	0
Query Match:	76.11%	Mismatches:	0
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US-10-009-782-1 (1-1758) x JC4394 (1-484)

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Qy      34 ATGTCCCAATCCGATTCCCAGCCCTTCGACCTGCTGCTCGCGGGCGGCACCCTCATCGAC 93
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Qy      94 GGCAGCAACACCCCGGGGCGGCGCGCCCGACCTGGGCGTGCGCGGCGACCGCATCGCCGCC 153
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Qy    154 ATCGGCGATCTGTCGGACGCCGCCGCGCACACCCGGGTCGACGTGTCGGGCCTGGTGGTC 213
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Qy    214 GCGCCCGGCTTCATCGACTCGCACACCCACGACGACAACTACCTGCTCAGGCGTCGCGAC 273
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     61 AlaProGlyPheIleAspSerHisThrHisAspAspAsnTyrLeuLeuArgArgArgAsp 80

Qy    274 ATGACGCCCAAGATCTCGCAGGGCGTCACCACGGTGGTCACGGGCAATTGCGGCATCAGC 333
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     81 MetThrProLysIleSerGlnGlyValThrThrValValThrGlyAsnCysGlyIleSer 100

Qy    334 CTGGCGCCGCTGGCGCACGCCAACCCGCCCGCCCCCCCTGGACCTGCTGGACGAAGGCGGC 393
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    101 LeuAlaProLeuAlaHisAlaAsnProProAlaProLeuAspLeuLeuAspGluGlyGly 120

Qy    394 TCTTACCGTTTCGAGCGCTTCGCCGACTACCTGGACGCGTTGCGGGCCACGCCGGCGGCC 453
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    121 SerTyrArgPheGluArgPheAlaAspTyrLeuAspAlaLeuArgAlaThrProAlaAla 140

Qy    454 GTCAACGCCGCCTGTATGGTGGGCCATTCAACGCTGCGCGCCGCGGTCATGCCGGACTTG 513
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    141 ValAsnAlaAlaCysMetValGlyHisSerThrLeuArgAlaAlaValMetProAspLeu 160

Qy    514 CAGCGCGCCGCCACCGACGAGGAAATCGCGGCCATGCGGGACCTGGCCGAGGAAGCCATG 573
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    161 GlnArgAlaAlaThrAspGluGluIleAlaAlaMetArgAspLeuAlaGluGluAlaMet 180

Qy    574 GCCAGCGGCGCCATCGGCATTTCGACCGGCGCCTTCTACCCGCCCGCCGCCCGCGCCACC 633
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    181 AlaSerGlyAlaIleGlyIleSerThrGlyAlaPheTyrProProAlaAlaArgAlaThr 200

Qy    634 ACCGAAGAGATCATCGAGGTGTGCCGGCCGCTGAGCGCGCATGGCGGCATCTACGCCACC 693
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    201 ThrGluGluIleIleGluValCysArgProLeuSerAlaHisGlyGlyIleTyrAlaThr 220

Qy    694 CACATGCGCGACGAAGGCGAGCACATCGTGGCCGCGCTGGAGGAAACCTTCCGCATCGGC 753
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    221 HisMetArgAspGluGlyGluHisIleValAlaAlaLeuGluGluThrPheArgIleGly 240

Qy    754 CGCGAGCTGGACGTGCCGGTGGTGATCTCGCACCACAAGGTCATGGGCCAGCCCAATTTC 813
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    241 ArgGluLeuAspValProValValIleSerHisHisLysValMetGlyGlnProAsnPhe 260

Qy    814 GGCCGCTCGCGCGAGACGCTGCCGCTGATCGAGGCCGCCATGGCGCGCCAGGACGTCTCG 873
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    261 GlyArgSerArgGluThrLeuProLeuIleGluAlaAlaMetAlaArgGlnAspValSer 280

Qy    874 CTGGACGCGTATCCCTACGTGGCCGGCTCCACCATGCTCAAGCAGGACCGCGTGCTGCTG 933
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Db    281 LeuAspAlaTyrProTyrValAlaGlySerThrMetLeuLysGlnAspArgValLeuLeu 300

Qy    934 GCCGGACGCACCATCATCACCTGGTGCAAGCCCTTCCCCGAACTGAGCGGGCGCGACCTG 993
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Db    301 AlaGlyArgThrIleIleThrTrpCysLysProPheProGluLeuSerGlyArgAspLeu 320

Qy    994 GATGAAGTCGCGGCCGAGCGCGGCAAATCCAAGTACGACGTGGTGCCCGAGCTGCAGCCG 1053
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    321 AspGluValAlaAlaGluArgGlyLysSerLysTyrAspValValProGluLeuGlnPro 340

Qy   1054 GCCGGCGCCATCTACTTCATGATGGACGAACCCGACGTGCAGCGCATCCTGGCGTTCGGC 1113
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Db    341 AlaGlyAlaIleTyrPheMetMetAspGluProAspValGlnArgIleLeuAlaPheGly 360

Qy   1114 CCGACCATGATCGGCTCCGACGGCCTGCCGCACGACGAGCGCCCGCATCCGCGCCTGTGG 1173
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Db    361 ProThrMetIleGlySerAspGlyLeuProHisAspGluArgProHisProArgLeuTrp 380

Qy   1174 GGCACCTTCCCGCGGGTGCTGGGGCACTATGCGCGCGACCTGGGCCTGTTCCCGCTGGAG 1233
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Db    381 GlyThrPheProArgValLeuGlyHisTyrAlaArgAspLeuGlyLeuPheProLeuGlu 400

Qy   1234 ACGGCGGTATGGAAGATGACCGGCCTGACCGCCGCGCGCTTCGGCCTGGCCGGGCGCGGG 1293
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Db    401 ThrAlaValTrpLysMetThrGlyLeuThrAlaAlaArgPheGlyLeuAlaGlyArgGly 420

Qy   1294 CAGCTGCAGGCCGGGTACTTCGCCGACCTGGTGGTGTTCGACCCGGCCACGGTGGCCGAT 1353
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Db    421 GlnLeuGlnAlaGlyTyrPheAlaAspLeuValValPheAspProAlaThrValAlaAsp 440

Qy   1354 ACCGCCACCTTCGAACACCCTACCGAGCGCGCCGCCGGCATCCATTCCGTGTACGTCAAC 1413
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Db    441 ThrAlaThrPheGluHisProThrGluArgAlaAlaGlyIleHisSerValTyrValAsn 460

Qy   1414 GGCGCGCCGGTCTGGCAAGAGCAGGCGTTCACCGGCCAGCATGCCGGCCGCGTGCTCGCA 1473
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Db    461 GlyAlaProValTrpGlnGluGlnAlaPheThrGlyGlnHisAlaGlyArgValLeuAla 480

Qy   1474 CGCACGGCCGCC 1485
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Db    481 ArgThrAlaAla 484

RESULT 2
JC4165
N-acyl-D-glutamate amidohydrolase (EC 3.5.1.-) - Alcaligenes xylosoxydans subsp. xy
C;Species: Alcaligenes xylosoxydans subsp. xylosoxydans A-6
C;Date: 12-Oct-1995 #sequence_revision 10-Nov-1995 #text_change 07-May-1999
C;Accession: JC4165
R;Wakayama, M.; Ashika, T.; Miyamoto, Y.; Yoshikawa, T.; Sonoda, Y.; Sakai, K.; Mor
J. Biochem. 118, 204-209, 1995
A;Title: Primary structure of N-acyl-D-glutamate amidohydrolase from Alcaligenes xyl
A;Reference number: JC4165; MUID:96015170; PMID:8537313
A;Accession: JC4165
A;Molecule type: DNA
A;Residues: 1-488 <WAK>
A;Cross-references: DDBJ:D45918
A;Note: The authors translated the codon CAG for residue 132 as Ala, GGC for residue
C;Comment: This enzyme catalyzes the hydrolysis of N-acyl derivatives of various D-a
C;Genetics:
A;Gene: dag
C;Superfamily: aminoacylase
C;Keywords: hydrolase

Alignment Scores:
Pred. No.:                8.09e-47      Length:          488
Score:                    1000.50       Matches:         223
Percent Similarity:       59.38%        Conservative:    62
Best Local Similarity:    46.46%        Mismatches:      190
Query Match:              30.33%        Indels:          5
DB:                       2             Gaps:            4

US-10-009-782-1 (1-1758) x JC4165 (1-488)

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Db      3 GluLysLeuAspLeuValIleGluGlyGlyTrpValIleAspGlyLeuGlyGlyProArg 22

Qy    112 CGGCGCGGCCGACCTGGGCGTGCGCGGCGACCGCATCGCCGCCATCGGCGATCTGTCGGAC 171
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Db     23 ArgArgAlaAspValGlyIleArgGlyGluArgIleAlaAlaIleGlyAspLeuSerAla 42

Qy    172 GCCGCCGCGCACACCCGGGTCGACGTGTCGGGCCTGGTGGTCGCGCCCGGCTTCATCGAC 231
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Db     43 AlaProAlaAspArgArgLeuAspAlaGlyGlyArgIleValAlaProGlyPheIleAsp 62

Qy    232 TCGCACACCCACGACGACAACTACCTGCTCAGGCGTCGCGACATGACGCCCAAGATCTCG 291
          :::|||   |||||||||          :::   :::       :::     |||   |||
Db     63 ThrHisGlyHisAspAspLeuMetPheValGluLysProGlyLeuGluTrpLysThrSer 82

Qy    292 CAGGGCGTCACCACGGTGGTCACGGGCAATTGCGGCATCAGCCTGGCGCCGCTG---GCG 348
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Db     83 GlnGlyIleThrSerValValValGlyAsnCysGlyIleSerGlyAlaProAlaProLeu 102

Qy    349 CACGCCAACCCGCCCGCCCCCCTGGACCTGCTGGACGAAGGCGGCTCTTACCGTTTCGAG 408
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Db    1141 CCGCACGACGAGCGCCCGCATCCGCGCCTGTGGGGCACCTTCCCGCGGGTGCTGGGGCAC 1200
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Db    1201 TATGCGCGCGACCTGGGCCTGTTCCCGCTGGAGACGGCGGTATGGAAGATGACCGGCCTG 1260
Qy    1261 ACCGCCGCGCGCTTCGGCCTGGCCGGGCGCGGGCAGCTGCAGGCCGGGTACTTCGCCGAC 1320
Db    1261 ACCGCCGCGCGCTTCGGCCTGGCCGGGCGCGGGCAGCTGCAGGCCGGGTACTTCGCCGAC 1320
Qy    1321 CTGGTGGTGTTCGACCCGGCCACGGTGGCCGATACCGCCACCTTCGAACACCCTACCGAG 1380
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Qy    1381 CGCGCCGCCGGCATCCATTCCGTGTACGTCAACGGCGCGCCGGTCTGGCAAGAGCAGGCG 1440
Db    1381 CGCGCCGCCGGCATCCATTCCGTGTACGTCAACGGCGCGCCGGTCTGGCAAGAGCAGGCG 1440
Qy    1441 TTCACCGGCCAGCATGCCGGCCGCGTGCTCGCACGCACGGCCGCCTGAGCCCGGCGCCAG 1500
Db    1441 TTCACCGGCCAGCATGCCGGCCGCGTGCTCGCACGCACGGCCGCCTGAGCCCGGCGCCAG 1500
Qy    1501 CCCTTACAATCCGGCGTGAACGGGGCGGCGTGCCGCCCCCTCCCAACCCTGGACGCAAAC 1560
Db    1501 CCCTTACAATCCGGCGTGAACGGGGCGGCGTGCCGCCCCCTCCCAACCCTGGACGCAAAC 1560
Qy    1561 CGCTACATGGCCCCTCCCTCCGCTCGCAATACGGCCCCACCCGATATCGTGGGCAAGGAA 1620
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Db    1621 GTGATGGGCGCGCGCCTGCGCGCCGAGCGCAAGGCCCGGAAAATGACCCTGCAAGACCTG 1680
Qy    1681 TCGCAGGCCAGCGGCATCGCGGTCTCGACCCTGTCCAAGGCCGAGCTGGGCCAGATCGCC 1740
Db    1681 TCGCAGGCCAGCGGCATCGCGGTCTCGACCCTGTCCAAGGCCGAGCTGGGCCAGATCGCC 1740
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Db    1741 CTGAGCTACGAGAAGCTT 1758
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Hind III

RESULT 4
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LOCUS S80683 1980 bp DNA linear BCT 13-AUG-1996
DEFINITION dan-D-aminoacylase [Alcaligenes xylosoxydans, ssp. xylosoxydans, A-6, Genomic, 1980 nt].
ACCESSION S80683
VERSION S80683.1 GI:1488356
KEYWORDS .
SOURCE Achromobacter xylosoxidans A-6 ssp. xylosoxydans.
ORGANISM Achromobacter xylosoxidans
Bacteria; Proteobacteria; beta subdivision; Alcaligenaceae; Achromobacter.
REFERENCE 1 (bases 1 to 1980)
AUTHORS Wakayama,M., Katsuno,Y., Hayashi,S., Miyamoto,Y., Sakai,K. and Moriguchi,M.
TITLE Cloning and sequencing of a gene encoding D-aminoacylase from Alcaligenes xylosoxydans subsp. xylosoxydans A-6 and expression of the gene in Escherichia coli
JOURNAL Biosci. Biotechnol. Biochem. 59 (11), 2115-2119 (1995)
MEDLINE 96100942
PUBMED 8541651
REMARK GenBank staff at the National Library of Medicine created this entry [NCBI gibbsq 174864] from the original journal article.
This sequence comes from Fig. 3.
FEATURES Location/Qualifiers
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/db_xref="taxon:85698"
gene 201. .1655
/gene="dan"

CDS 201. .1655
/gene="dan"
/note="3.5.1.14; Method: conceptual translation with partial peptide sequencing; This sequence comes from Fig. 3; N-acyl-D-amino acid amidohydrolase"
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/transl_table=11
/product="D-aminoacylase"
/protein_id="AAB35881.1"
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ATHMRDEGEHIVAALEETFRIGRELDVPVVISHHKVMGQPNFGRSRETLPLIEAAMAR
QDVSLDAYPYVAGSTMLKQDRVLLAGRTIITWCKPFPELSGRDLDEVAAERGKSKYDV
VPELQPAGAIYFMMDEPDVQRILAFGPTMIGSDGLPHDERPHPRLWGTFPRVLGHYAR
DLGLFPLETAVWKMTGLTAARFGLAGRGQLQAGYFADLVVFDPATVADTATFEHPTER
AAGIHSVYVNGAPVWQEQAFTGQHAGRVLARTAA"
BASE COUNT 298 a 758 c 642 g 282 t
ORIGIN

Eco RI

Query Match 98.9%; Score 1739.4; DB 1; Length 1980;
Best Local Similarity 99.4%; Pred. No. 9.1e-181;
Matches 1746; Conservative 0; Mismatches 11; Indels 0; Gaps 0;

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Qy       1 GAATTCCACTTGATCGCGGAAGGAGAGATTTCCATGTCCCAATCCGATTCCCAGCCCTTC 60
Db     168 GATCGCCACTTGATCGCCCCGCCGGAGATTTCCATGTCCCAATCCGATTCCCAGCCCTTC 227
Qy      61 GACCTGCTGCTCGCGGGCGGCACCCTCATCGACGGCAGCAACACCCCGGGGCGGCGCGCC 120
Db     228 GACCTGCTGCTCGCGGGCGGCACCCTCATCGACGGCAGCAACACCCCGGGGCGGCGCGCC 287
Qy     121 GACCTGGGCGTGCGCGGCGACCGCATCGCCGCCATCGGCGATCTGTCGGACGCCGCCGCG 180
Db     288 GACCTGGGCGTGCGCGGCGACCGCATCGCCGCCATCGGCGATCTGTCGGACGCCGCCGCG 347
Qy     181 CACACCCGGGTCGACGTGTCGGGCCTGGTGGTCGCGCCCGGCTTCATCGACTCGCACACC 240
Db     348 CACACCCGGGTCGACGTGTCGGGCCTGGTGGTCGCGCCCGGCTTCATCGACTCGCACACC 407
Qy     241 CACGACGACAACTACCTGCTCAGGCGTCGCGACATGACGCCCAAGATCTCGCAGGGCGTC 300
Db     408 CACGACGACAACTACCTGCTCAGGCGTCGCGACATGACGCCCAAGATCTCGCAGGGCGTC 467
Qy     301 ACCACGGTGGTCACGGGCAATTGCGGCATCAGCCTGGCGCCGCTGGCGCACGCCAACCCG 360
Db     468 ACCACGGTGGTCACGGGCAATTGCGGCATCAGCCTGGCGCCGCTGGCGCACGCCAACCCG 527
Qy     361 CCCGCCCCCCCTGGACCTGCTGGACGAAGGCGGCTCTTACCGTTTCGAGCGCTTCGCCGAC 420
Db     528 CCCGCCCCCCCTGGACCTGCTGGACGAAGGCGGCTCTTACCGTTTCGAGCGCTTCGCCGAC 587
Qy     421 TACCTGGACGCGTTGCGGGCCACGCCGGCGGCCGTCAACGCCGCCTGTATGGTGGGCCAT 480
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Qy     481 TCAACGCTGCGCGCCGCGGTCATGCCGGACTTGCAGCGCGCCGCCACCGACGAGGAAATC 540
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Qy     541 GCGGCCATGCGGGACCTGGCCGAGGAAGCCATGGCCAGCGGCGCCATCGGCATTTCGACC 600
Db     708 GCGGCCATGCGGGACCTGGCCGAGGAAGCCATGGCCAGCGGCGCCATCGGCATTTCGACC 767
Qy     601 GGCGCCTTCTACCCGCCCGCCGCCCGCGCCACCACCGAAGAGATCATCGAGGTGTGCCGG 660
Db     768 GGCGCCTTCTACCCGCCCGCCGCCCGCGCCACCACCGAAGAGATCATCGAGGTGTGCCGG 827
Qy     661 CCGCTGAGCGCGCATGGCGGCATCTACGCCACCCACATGCGCGACGAAGGCGAGCACATC 720
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Db    828 CCGCTGAGCGCGCATGGCGGCATCTACGCCACCCACATGCGCGACGAAGGCGAGCACATC 887

Qy    721 GTGGCCGCGCTGGAGGAAACCTTCCGCATCGGCCGCGAGCTGGACGTGCCGGTGGTGATC 780
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Db    888 GTGGCCGCGCTGGAGGAAACCTTCCGCATCGGCCGCGAGCTGGACGTGCCGGTGGTGATC 947

Qy    781 TCGCACCACAAGGTCATGGGCCAGCCCAATTTCGGCCGCTCGCGCGAGACGCTGCCGCTG 840
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Qy    841 ATCGAGGCCGCCATGGCGCGCCAGGACGTCTCGCTGGACGCGTATCCCTACGTGGCCGGC 900
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Qy    901 TCCACCATGCTCAAGCAGGACCGCGTGCTGCTGGCCGGACGCACCATCATCACCTGGTGC 960
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Qy    961 AAGCCCTTCCCCGAACTGAGCGGGCGCGACCTGGATGAAGTCGCGGCCGAGCGCGGCAAA 1020
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Qy   1021 TCCAAGTACGACGTGGTGCCCGAGCTGCAGCCGGCCGGCGCCATCTACTTCATGATGGAC 1080
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Db   1908 CTGAGCTACGAGAAGTT 1924
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HindIII

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DEFINITION  Alcaligenes xylosoxidans DNA for N-acyl-D-amino acid
            amidohydrolase, complete cds.
ACCESSION   D45918
VERSION     D45918.1  GI:1255443
KEYWORDS    N-acyl-D-amino acid amidohydrolase.
SOURCE      Alcaligenes xylosoxidans (sub_species:xylosoxydans, isolate:A-6)
            DNA.
  ORGANISM  Achromobacter xylosoxidans
            Bacteria; Proteobacteria; beta subdivision; Alcaligenaceae;
            Achromobacter.
REFERENCE   1  (sites)
  AUTHORS   Wakayama,M., Ashika,T., Miyamoto,Y., Yoshikawa,T., Sonoda,Y.,
            Sakai,K. and Moriguchi,M.
  TITLE     Primary structure of N-acyl-D-glutamate amidohydrolase from
            Alcaligenes xylosoxydans subsp. xylosoxydans A-6
  JOURNAL   J. Biochem. 118 (1), 204-209 (1995)
  MEDLINE   96015170
REFERENCE   2  (sites)
  AUTHORS   Wakayama,M., Katsuno,Y., Hayashi,S., Miyamoto,Y., Sakai,K. and
            Moriguchi,M.
  TITLE     Cloning and sequencing of a gene encoding D-aminoacylase from
            Alcaligenes xylosoxydans subsp. xylosoxydans A-6 and expression of
            the gene in Escherichia coli
  JOURNAL   Biosci. Biotechnol. Biochem. 59 (11), 2115-2119 (1995)
  MEDLINE   96100942
REFERENCE   3  (bases 1 to 1455)
  AUTHORS   Wakayama,M.
  JOURNAL   Unpublished
REFERENCE   4  (bases 1 to 1455)
  AUTHORS   Wakayama,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-FEB-1995) Mamoru Wakayama, Oita University, Applied
            Chemistry; Dannoharu, Oita, Oita 870-11, Japan
            (Tel:0975-69-3311(ex.746), Fax:0975-69-7957)
FEATURES             Location/Qualifiers
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                     DLGLFPLETAVWKMTGLTAARFGLAGRGQLQAGYFADLVVFDPATVADTATFEHPTER
                     AAGIHSVYVNGAPVWQEQAFTGQHAGRVLARTAA"
BASE COUNT      222 a    552 c    477 g    204 t
ORIGIN

  Query Match           82.7%;  Score 1453.4;  DB 1;  Length 1455;
  Best Local Similarity  99.9%;  Pred. No. 1.3e-149;
  Matches 1454;  Conservative    0;  Mismatches    1;  Indels    0;  Gaps    0;

Qy      34 ATGTCCCAATCCGATTCCCAGCCCTTCGACCTGCTGCTCGCGGGCGGCACCCTCATCGAC 93
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Db       1 ATGTCCCAATCCGATTCCCAGCCCTTCGACCTGCTGCTCGCGGGCGGCACCCTCATCGAC 60

Qy      94 GGCAGCAACACCCCGGGGCGGCGCGCCGACCTGGGCGTGCGCGGCGACCGCATCGCCGCC 153
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Table 20-2 Some restriction endonucleases and their cleavage sites

Microorganism	Name of enzyme	Target sequence and cleavage sites
Generates cohesive ends		
E. coli	EcoRI	G↓A A T T C C T T A A↑G
Bacillus amyloliquefaciens H	BamHI	G↓G A T C C C C T A G↑G
B. globigii	BglII	A↓G A T C T T C T A G↑A
Haemophilus aegyptius	HaeII	Pu G C G C↓Py Py↑C G C G Pu
Haemophilus influenza	HindIII	A↓A G C T T T T C G A↑A
Providencia stuartii	PstI	C T G C A↓G G↑A C G T C
Streptococcus albus G	SalI	G↓T C G A C C A G C T↑G
Thermus aquaticus	TaqI	T↓C G A A G C↑T
Generates flush ends		
Brevibacterium albidum	BalI	T G G↓C C A A C C G G T
Haemophilus aegyptius	HaeI	(A/T) G G↓C C (T/A) (T/A) C C G G (A/T)
Serratia marcescens	SmaI	C C C↓G G G G G G C C C

Note: The vertical dashed line indicates the axis of dyad symmetry in each sequence. Arrows indicate the sites of cutting. The enzyme TaqI yields cohesive ends consisting of two nucleotides, whereas the cohesive ends produced by the other enzymes contain four nucleotides. The enzyme HaeI recognizes the sequence GGCC whether the adjacent base pair is A·T or T·A, as long as dyad symmetry is retained. Pu and Py refer to any purine and pyrimidine, respectively.